

AMENDMENTS TO THE SPECIFICATION

Please amend the paragraphs and Tables: Table 1, [0038], [0039], [0040], [0041], [0046], [0052], Table 2, Table 3, Table 4, Table 5, [0071], Table 7, and [00184] as follows:

Table 1.

TABLE 1

AA RESIDUES TO USE TO PERFORM RMSD COMPARISON	PORTION OF EACH AA RESIDUE USED TO PERFORM RMSD COMPARISON	RMSD VALUE LESS THAN OR EQUAL TO		
Table 2 (4 Angstrom set) (relative to S630)	alpha-carbon atoms ¹	0.75	0.50	0.38
	main-chain atoms ¹	0.82	0.55	0.41
	all non-hydrogen ²	0.95	0.64	0.48
Table 3 (7 Angstrom set) (relative to S630)	alpha-carbon atoms ¹	3.84	2.56	1.92
	main-chain atoms ¹	3.83	2.55	1.91
	all non-hydrogen ²	4.15	2.77	2.07
Table 4 (10 Angstrom set) (relative to S630)	alpha-carbon atoms ¹	4.25	2.83	2.13
	main-chain atoms ¹	4.29	2.86	2.14
	all non-hydrogen ²	4.60	3.07	2.30
<u>Residues 39-757 of</u> SEQ. ID No. 1	alpha-carbon atoms ¹	6.96	4.64	3.48
	main-chain atoms ¹	6.92	4.61	3.46
	all non-hydrogen ²	7.08	4.72	3.54

¹- the RMSD computed between the atoms of all amino acids that are common to both the target and the reference in the aligned and superposed structure. The amino acids need not to be identical.

²- the RMSD computed only between identical amino acids, which are common to both the target and the reference in the aligned and superposed structure.

[0038] Figure 2 illustrates a crystal of FAP α complex (SEQ. ID No. 3).

[0039] Figure 3 lists a set of atomic structure coordinates for FAP α (SEQ. ID No. 1) as derived by X-ray crystallography from a crystal that comprises the protein. The following abbreviations are used in Figure 3: "X, Y, Z" crystallographically define the atomic position of the element measured; "B" is a thermal factor that measures movement of the atom around its atomic center; "Occ" is an occupancy factor that refers to the fraction of the molecules in which each atom

occupies the position specified by the coordinates (a value of "1" indicates that each atom has the same conformation, i.e., the same position, in all molecules of the crystal).

[0040] Figure 4 illustrates a ribbon diagram overview of the structure of ~~FAP~~ FAP α , highlighting secondary structural elements of the protein.

[0041] Figure 5 illustrates the FAP α binding site of FAP α based on the determined crystal structure for the molecule in the asymmetric unit corresponding to the coordinates shown in Figure 3 (SEQ. ID No. 1).

[0046] It should be understood that the methods and compositions provided relating to ~~FAP~~ FAP α are not intended to be limited to the wild type, full length form of FAP α . Instead, the present invention also relates to fragments and variants of FAP α as described herein. Further, the present invention has applicability to other S9 proteases whose sequence and/or structure are comparatively similar to FAP α .

[0052] For example, based on the crystal structure, applicants have determined that the ~~FAP~~ FAP α amino acids shown in Table 1 encompass a 4-Angstrom radius around the FAP α active site and thus likely to interact with any active site inhibitor of FAP α . Applicants have also determined that the amino acids of Table 2 encompass a 7-Angstrom radius around the FAP α active site. Further it has been determined that the amino acids of Table 3 encompass a 10-Angstrom radius around the FAP α active site. It is noted that there are two different FAP α molecules in the asymmetric unit, referred to as chains A and B. As a result, two sets of structure coordinates were obtained for each amino acid. There is one dimer formed in the asymmetric unit between molecules A and B. Applicants have also determined that amino acids of Table 5 encompass a 5-Angstrom radius around the FAP α amino acids that interact at the AB dimerization interface. The A and B sets of structural coordinates appear in Figure 3. It is noted that the sequence and structure of the residues in the active site and dimerization interface may also be conserved and hence pertinent to other S9 proteases.

Table 2: Amino Acids encompassed by a 4-Angstrom radius around the FAP α active site (SEQ. ID No. 1).

Trp623	Gly627	Pro649
Ser624	Tyr628	Val650
Tyr625	Val647	Val705
Gly626	Ala648	His734

Table 3: Amino Acids encompassed by a 7-Angstrom radius around the FAP α active site (SEQ. ID No. 1).

Val540	Gly627	Val650
Tyr541	Tyr628	Ser651
Gly622	Val629	Trp653
Trp623	Ala646	Tyr656
Ser624	Val647	Asn704
Tyr625	Ala648	Val705
Gly626	Pro649	His734

Table 4: Amino Acids encompassed by a 10-Angstrom radius around the FAP α active site (SEQ. ID No. 1).

Arg123	Gly627	Tyr694
Trp199	Tyr628	Leu696
Glu203	Val629	Ile697
Glu204	Ser630	His698
Tyr252	Ser631	Gly699
Phe350	Leu632	Asp702
Val540	Ile645	Asn704
Tyr541	Ala646	Val705
Gly542	Val647	His706
Gly543	Ala648	Asn709
Pro544	Pro649	Ser710
Glu596	Val650	Ile713
Trp621	Ser651	Asn733
Gly622	Ser652	His734
Trp623	Trp653	Gly735
Ser624	Tyr656	Leu736
Tyr625	Tyr660	Leu744
Gly626	Val682	Met748

Table 5: Amino Acids encompassed by a 5-Angstrom radius around the AB dimerization interface (SEQ. ID No. 1).

Chain A	Chain B
Pro232	Pro232
Val233	Val233
Ile234	Ile234
Ala235	Ala235
Tyr236	Tyr236
Ser237	Ser237
Tyr239	Tyr239
Gly240	Gly240
Asp241	Asp241
Glu242	Glu242
Gln243	Gln243
Tyr244	Tyr244
Pro245	Pro245
Thr247	Thr247
Tyr252	Tyr252
Pro253	Pro253
Lys254	Lys254
Ala255	Ala255
Glu654	Glu654
Tyr655	Tyr655
Leu696	Leu696
Phe707	Phe707
Gln708	Gln708
Ser710	Ser710
Ala711	Ala711
Gln712	Gln712
Ala714	Ala714
Lys715	Lys715
Leu717	Leu717
Val718	Val718
Asn719	Asn719
Gln721	Gln721
Val722	Val722
Asp723	Asp723
Phe724	Phe724
Gln725	Gln725
Ala726	Ala726
Met727	Met727
Trp728	Trp728
Ser730	Ser730

Ser731	Ser731
Leu739	Leu739
Ser740	Ser740
Asn742	Asn742
His743	His743
His747	His747
His750	His750

2. Cloning, Expression and Purification of FAP $\square$ FAP α

[0071] The gene encoding FAP α can be isolated from RNA, cDNA or cDNA libraries. In this case, the portion of the gene encoding amino acid residues 27-760 (SEQ. ID No. 1), corresponding to the catalytic domain of human FAP α , was isolated and is shown as SEQ. ID No. 2.

Table 7.

TABLE 7

Crystal data		
Space group		P2 ₁ 2 ₁ 2 ₁
Unit cell dimensions		a=70.31Å b=152.60Å c=214.65Å
Data collection		
X-ray source		ALS BL 5.0.3
Wavelength [Å]		1.00
Resolution [Å]		2.30
Observations (unique)		89676
Redundancy		2.5
Completeness	overall (outer shell)	89(64)%
$\frac{I}{\sigma(I)}$	overall (outer shell)	7.8 (1.8)
R_{symm}	overall (outer shell)	11.7 (35.2)%
Refinement		
Reflections used		84874
R-factor		24.4%
R_{free}		29.1%
r.m.s bonds		0.03Å
r.m.s angles		2.7°

[00184] Following harvest, the cell cultures were centrifuged to pellet whole cells and the culture medium supernatant was concentrated using a cross-flow ultrafiltration apparatus. In a typical batch preparation, 5 L of cell culture supernatant were concentrated to 0.1 L on a 10 kDa NMWCO Omega Ultrasette (Pall Life Sciences) using a Masterflex L/S pump fitted with PharMed #15 tubing at a cross flow of approximately 1 L/min and an inlet feed pressure of 1.5 to 2.0 bar, generating an initial permeate flow of up to 70 ml/min. The retentate was diluted two to three-fold by adding 25 mM Tris/HCl pH 7.9, 0.4 M NaCl and reconcentrated to 0.1 L. This process was repeated at least twice, after which the concentrate was quantitatively removed from the system, centrifuged when necessary (15 minutes at 4,000 rpm in an Allegra (Beckman) centrifuge) and added to approximately 8 ml of a preconditioned 50 % slurry of Probond (Invitrogen) divided over three or four 50ml conical tubes. The tubes were rotated for at least 1 hour, after which the resin was washed with 10 resin volumes of 50 mM Potassium Phosphate pH 7.9, 0.4 M NaCl, 0.25 mM [[TCEP]] Tris(2-carboxyethyl)phosphine hydrochloride (TCEP). The resin was poured into 1 cm ID glass columns (Omnifit) and washed with 50 column volumes of 50 mM Potassium Phosphate pH 7.9, 0.4 M NaCl, 20 mM imidazole, 0.25 mM TCEP. After a wash with 5 column volumes of 50 mM Tris pH 7.9, 0.4 M NaCl, 0.25 mM TCEP, the product is eluted with 4 column volumes of 50 mM Tris pH 7.9, 0.4 M NaCl, 200 mM imidazole, 0.25 mM TCEP. It is noted that for the purification of non-secreted proteins, leupeptin is added to all the buffers used during the IMAC process at 1 mg/L and that for simplicity reasons the same is sometimes done when purifying FAP α .